REMARKS

This is responsive to the Advisory Action mailed December 30, 2010. Applicants thank the Examiner for entry of their December 15, 2010 Amendment, and for withdrawal of the 35 U.S.C. §112, first paragraph, rejection. By this Amendment and Response, Claim 56 is amended, new claim 118 is added, and Claims 56-64, 93, 100, 103-106, and 110-118 are pending for examination. Entry of this amendment is respectfully requested. It is submitted that the rejections are overcome in view of the amendments and remarks presented herein, and that the application is in condition for allowance. Favorable reconsideration of the application is respectfully requested.

I. AMENDMENT TO THE CLAIMS

Support for the newly added language of Claim 56, "wherein the first promoter is a strong promoter," is found, for example, at paragraphs 39 and 117 of the specification.

> Yet another aspect of the invention provides mammalian host cells comprising a first cistron encoding a transactivator, a second cistron encoding an apoptosis-protective protein that prevents cell-killing due to expression of the transactivator, and a third cistron encoding one or more desired proteins under the control of a promoter responsive to the transactivator. In a preferred embodiment, the transactivator is expressed from ***an efficient heterologous promoter*** at a level that, in the absence of the protective protein, causes significant cell death. In some embodiments, the invention provides a non-human mammalian host cell. An aspect of the invention also provides methods for producing a recombinant protein comprising culturing the mammalian host cells in a suitable medium such that the desired protein(s) is secreted into the medium.

(Specification at ¶ 39 (emphasis added).)

> Various vectors and methods for introducing them into host cells are available for obtaining appropriate expression of the transactivator and the protective cistron of the invention. For example the cistrons can each be expressed from ***a strong constitutive promoter*** such as the ***hCMV-MIE promoter*** in plasmids pCI-neo or pCDNA3, or from a ***RSV-LTR promoter***. The cistrons can be on the same or separate plasmids and appropriate selectable markers are used to select for clones in which the vectors have integrated into the genome. For example markers that confer resistance to hygromycin, neomycin (G418) or zeocin can be used. The cistron or cistrons used to express the recombinant protein may also be introduced on the same or a different plasmid and can be introduced using various selectable markers including resistance markers or amplifiable markers such as GS or

> DHFR. Various additional elements that are known in the art may be included with the transactivator cistron, the protective cistron or the activatable cistron to ensure appropriate levels of expression. For example splice sites, polyadenylation signals, 5' or 3' untranslated regions may be added. For the activatable cistron, efficient expression is desired and additional elements intended to enhance expression levels may be added, including UCOEs, insulators, barrier elements and signal peptides. The vectors for expression of the cistrons are introduced into the mammalian host cell by any of the available methods including calcium phosphate co-precipitation, electroporation or cationic lipid mediated transfection.

(Specification at ¶ 117 (emphasis added).) Thus, the specification supports a strong promoter for expression of the transactivator. Paragraph 39 discusses the use of efficient promoters to express the transactivator, and paragraph 117 discusses the use of strong constitutive promoters such as hCMV-MIE and RSV-LTR promoters which are known to the person of ordinary skill in the art as strong promoters.

New Claim 118 is a combination of Claims 56 and 111 as presented in the Amendment and Response filed on December 15, 2010.

II. REJECTIONS UNDER 35 U.S.C. §103

Claims 56-64, 93, 100, 103-106, and 110-117 are rejected under 35 U.S.C. §103(a) as being unpatentable over Reff et al. (IDS), Cockett et al. (IDS) and Rao et al. (PNAS, 1992. Vol. 89, pages 7742-7746) in view of Antoniou et al. (WO 00/05393). The Applicants respectfully traverse these rejections because the cited prior art (1) fails to teach all the limitations of the claims, (2) teaches away from the invention as claimed, and (3) fails to provide a reasonable expectation of success.

Independent Claims 56, 62, 93, and 100 claim host cells and methods of making recombinant protein where a transactivator for the desired protein is expressed at a level that can cause cell death, and this toxicity is countered by an apoptosis-protective protein that is expressed at levels to inhibit cell death caused by the transactivator. Claim 56 has been amended to recite that the transactivator is expressed from a strong promoter. In addition, Claims 110-117 add limitations to these independent claims that require an increase in productivity of 2-5 fold from the transactivator and apoptosis-protective protein. New Claim 118 claims a method of

making a recombinant protein where a transactivator for the desired protein is expressed at a level that can cause cell death, this toxicity is countered by an apoptosis-protective protein that rescues the host cells from cell death which would be caused by the transactivator, and this combination of transactivator and apoptosis protective protein enhances recombinant protein production by at least 5-fold. These limitations are not disclosed in Reff, Cockett, Rao and/or Antoniou, and it would not be obvious to add these limitations to the combination of Reff, Cockett, Rao and/or Antoniou.

Reff teaches that apoptosis will naturally occur in the life cycle of host cells recombinantly expressing a desired polypeptide, when those host cells grow to a density such that the host cells are exposed to external factors such as nutrient limitation (*e.g.*, serum deprivation), toxic agents in the growth media (e.g., waste products from cell growth), or physical stresses. (*See* Reff at ¶¶ 9, 13-14, 25-31, 34-35, 42-43, 76-87, and 98-123.) Reff demonstrates enhanced recombinant protein production (*e.g.*, recombinant cultures can grow to a higher density where more cells are making the desired protein) when host cell life is extended beyond these limiting factors through the use of an apoptosis protective protein that prevents or delays apoptosis caused by these factors. (*Id.*) In view of the objects of the Reff invention, it would be detrimental to the host cell to add a toxic factor that would increase cell death (apoptosis), such as the claimed toxic levels of a transactivator produced by expression from a strong promoter. Introduction of such a toxicity factor would be expected to reduce cell growth/density reducing the production of recombinant protein as taught by Reff. Thus, it would not be obvious to obviate the objects of the Reff reference by combining it with toxic levels of transactivator protein as recited in the amended claims. Reff also provides no insights as to whether the claimed combination of toxic transactivator and rescuing apoptosis protective protein would enhance recombinant protein production as recited in Claims 110-118.

In addition, Reff teaches increased recombinant protein production by longer host cell lifetime (each cell produces for a longer time), and greater host cell density (the overall culture produces more protein per liter). In contrast, the pending claims enhance the amount of

recombinant protein produced by increasing the rate of production per cell (each cell produces protein at a faster rate) using toxic levels of a transactivator expressed from a strong promoter combined with rescue through an apoptosis protective protein. This is a different way of enhancing recombinant protein production compared to Reff.

Cockett teaches that high levels of transactivator inhibited the growth of host cells and that high levels of transactivator are undesirable for recombinant protein expression. (*See* abstract.) Cockett enhanced recombinant protein production using a weak promoter to express low levels of E1A rather than a strong promoter to express high levels of E1A. (See Table 2.) This teaching of a weak promoter and low levels of E1A are inconsistent with the amended claims recitation of a strong promoter for expressing toxic amounts of transactivator.

Cockett attributes the poor performance with high levels of transactivator to cell toxicity. This buttresses the teachings of Reff et al. that cell toxicity should be avoided because it is undesirable for recombinant protein expression. Cockett and Reff together teach that factors causing cell toxicity are to be avoided and this teaches away from the pending claims which intentionally introduce into the recombinant production system a factor causing toxicity, i.e., toxic levels of transactivator produced from a strong promoter.

At best, Reff combined with Cockett would teach that the host cells of Reff should be combined with low (non-toxic) levels of a transactivator expressed from a weak promoter to achieve recombinant protein expression. It would not be obvious from Cockett to modify Reff with toxic levels of a transactivator expressed from a strong promoter and then rescue those cells with an apoptosis protective protein. Neither of these references teach this should be done or provide any basis to reasonably predict (or expect) what the impact of this toxicity and rescue would have on recombinant protein expression. In fact, both Reff and Cockett teach that toxic levels of transactivator should be avoided. Thus, Reff and Cockett, the two cited references relevant to recombinant protein expression, do not teach toxic levels of transactivator expressed from a strong promoter for inducing expression of a desired protein, or rescuing such

recombinant production using an apoptosis protective protein, or a reasonable expectation of success that such recombinant protein expression would enhance protein production.

Since neither Reff nor Cockett teach toxic levels of transactivator expressed from a strong promoter for recombinant protein expression, or rescue with an apoptosis protective protein, the combination cannot teach the limitations of claims 110-118, that recombinant protein production is enhanced 2-5 fold by this combination of transactivator and apoptosis protective protein.

Rao does not overcome the shortcomings of Reff and Cockett because Rao does not teach recombinant protein expression. Instead, Rao teaches that primary cultures of rat kidney cells can be converted to a transformed cell type (a cancer like cell) by the E1A protein, but after an initial burst of growth many of these cancer-like cells die (by an apoptosis like process), and immortalized clones will arise from these E1A transformed primary cells after 5-6 weeks. (*See* abstract, and p. 7743, first col., first full paragraph.) Rao also teaches that during transformation the E1B protein can prevent the death of many of the E1A expressing cells after the initial burst of cell growth. (*Id.*) Thus, Rao is not directed to recombinant protein expression and a person of skill in the art would not combine Rao (making cancer like cells) with Reff and Cockett (recombinant protein expression).

In addition, Rao does not teach the limitations of the claim which are missing from Reff and Cockett. Rao does not teach the use of toxic levels of transactivator produced from a strong promoter to induce recombinant protein expression, or rescue of cells with such toxic levels of transactivator using an apoptosis protective protein during recombinant protein expression. Since Rao does not teach such recombinant production, Rao cannot teach enhancing recombinant protein expression 2-5 fold with this combination of toxic transactivator levels and rescue with an apoptosis protective protein. The combination of these three references does not teach all the limitations of the pending claims, and so, the Applicants respectfully submit this rejection is overcome.

In addition, a person of skill in the art would not have a reasonable expectation of success for the claimed invention based on Rao, Reff and Cockett. Reff and Cockett do not provide a reasonable expectation of success (and in fact teach away) for the reasons stated above. Rao does not cure this defect. Rao was studying immortalization of primary cell cultures and not recombinant protein expression. Nothing in Rao teaches that E1A or E1B can be used for recombinant protein expression, or that the results with E1A and E1B in this cancer induction model has any relationship to recombinant protein production. No art has been cited to bridge this gap between recombinant protein expression as claimed in the pending claims and induction of a cancer like state in primary cells using E1A and E1 as taught by Rao. Thus, a person of skill in the art would not have a reasonable expectation of success based on the combination of Reff, Cockett and Rao, *i.e.*, that expression of toxic levels of transactivator with rescue by an apoptosis-protective protein would work for recombinant protein expression, or that such a combination would enhance recombinant protein expression 2-5 fold. For these same reasons, Claim 118 is patentable over Reff, Cockett and Rao because this claim recites that the transactivator is expressed at toxic levels which are rescued by the apoptosis protective protein so that expression of the desired protein is enhanced at least 5 fold. Reff, Cockett and Rao do not teach all these limitations, and provide no reasonable expectation that the combination of these elements would enhance expression by at least 5 fold.

Antoniou was cited for teaching UCOE and IRES elements in an expression vector. These teachings do not bridge the gaps in Reff, Cockett and Rao, regarding expression of toxic levels of transactivator for inducing recombinant expression with rescue by an apoptosis-protective protein. Since Reff, Cockett and Rao do not teach such recombinant protein production, this combination cannot teach that such a combination will enhance recombinant production by 2-5 fold.

The case presented by the pending claims and Reff, Cockett and Rao is very similar to the Federal Circuit's decisions in *Eisai* and *Kinetic Concepts*. In *Eisai*, the Federal Circuit rejected an obviousness combination of prior art because the combination required that a feature

the prior art found as advantageous be dropped to make the claimed invention. *See Eisai Co. Ltd. v. Dr. Reddy's Lab., Ltd*, 87 USPQ2d 1452, 1456 (Fed. Cir. 2008) ("The record, however, shows no discernible reason for a skilled artisan to begin with lansoprazole only to drop the very feature, the fluorinated substituent, that gave this advantageous property.") As discussed above, Reff and Cockett together extol the benefits of reducing toxicity to host cells, including apoptosis, during recombinant production of desired proteins. Adding toxic levels of transactivator to Reff and Cockett would be contrary to the teachings of Reff and Cockett and would require that a person of skill in the art forego the advantageous feature taught by these references. *I.e.*, the object of the Reff publication is to reduce natural apoptosis and it would be antithetical to that goal to artificially introduce to the host cell factors that would increase apoptosis over the natural factors. Cockett bolsters this by its teachings that toxic levels of transactivator are undesirable for recombinant protein expression. Combining Rao with Reff and Cockett causes the very incongruity relied upon by the Federal Circuit in *Eisai*, it requires that the person of skill introduce into Reff and Cockett the very thing these references teach should be avoided, toxicity to the host cell.

In *Kinetic Concepts*, none of the asserted prior art taught "treating a wound with negative pressure" and so the Federal Circuit affirmed the District Court's holding of nonobviousness. *See Kinetic Concepts, Inc. v. Blue Sky Med. Grp, Inc.*, 554 F.3d 1010 (Fed. Cir. 2010); *see also, Honeywell Int'l, Inc. v. U.S.*, 93 USPQ2d 1740, 1747 (Fed. Cir. 2010) (prior art failed to teach perceptible red light as required by the claim). *Id.* The Federal Circuit held these claims nonobvious because the prior art failed to teach limitations of the claim. Similarly here, Reff, Cockett and Rao do not teach recombinant expression in a host cell using toxic levels of transactivator with rescue by an apoptosis protective protein. And, Reff Cockett and Rao do not teach that this combination increases recombinant protein production by 2-5 fold.

For all the above reasons, Applicant respectfully submits that the pending claims overcome the obviousness rejections combining Reff with Cockett, Rao and Antoniou.

III. CONCLUSION

Applicants believe that this application is in condition for allowance, and request that the Examiner give the application favorable reconsideration and permit it to issue as a patent. If the Examiner believes that the application can be put in even better condition for allowance, the Examiner is invited to contact Applicant's representatives listed below.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,

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